A robotic platform to assess, guide and perturb rat forelimb movements

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A robotic approach to investigate motor learning during rat forelimb movements

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Abstract

Animal models are widely used to explore the mechanisms underlying sensorimotor control and learning. However, current experimental paradigms allow only limited control over task difficulty and cannot provide detailed information on forelimb kinematics and dynamics. Here we propose a novel robotic approach to investigate motor learning in rats. The compact, highly transparent three degree-of-freedom manipulandum is capable of rendering forces up to 2*N* to guide or perturb rat forelimb movements, while providing objective and quantitative assessments of endpoint motor performance in a 50x30mm^2 planar workspace. Preliminary experiments with 10 healthy rats show that the animals can familiarize with the experimental setup, learn how to grasp and manipulate the end-effector, and that their motor behavior can be influenced by dynamic perturbations or external haptic guidance. Training in a haptic tunnel resulted in a significant reduction of the integrated straight line error (79.7%, $p<0.01$), which persisted after removal of the force field. This approach opens up new research avenues for future investigations of motor learning stages, both in healthy and in stroke models.

1. Introduction

Sensory-motor learning can be generally defined as the improvement of sensory-guided motor behaviour in response to practice (Krakauer and Mazzoni 2011). However, several distinct learning conditions contribute to this phenomenon: the successive acquisition of novel movement sequences that lead towards an asymptotic level of performance (i.e. motor skill learning; Reis et al. 2009) must be distinguished from regaining the baselineperformance of an already acquired task in presence of a perturbed motor environment (i.e. motor adaptation; Shadmehr and Wise 2005).

In rodent models of motor skill learning, a pellet-reaching task that requires the extension of the forepaw to reach for a food reward placed onto a pedestal (Whishaw and Pellis 1990) is frequently used to assess changes within the brain matrix in response to training (Monfils et al. 2005). While being well described and reproducible (Luft and Buitrago 2005), the value of this task is restricted due to the absence of recorded movement kinematics and dynamics, the limited ability to vary the complexity of the motor task, as well as by the large amount of time that is needed for the individual training of rats. Furthermore, as there is no possibility to perturb forelimb movements, the neurobiological bases of motor adaptation cannot be studied.

In humans and non-human primates, these limitations were resolved through the application of robotic devices allowing quantitative assessments of movement kinematics and dynamics, as well as the well-controlled and repeatable rendering of external dynamics (force fields) that precisely perturb movements, thereby challenging motor adaptation (Shadmehr and Mussa-Ivaldi 1994, Scott 1999, Graham et al. 2003). Although this approach allows the investigation of underlying internal models, the detection of functional changes within the human brain is limited to non-invasive imaging methods (Shadmehr and Holcomb 1997, Diedrichsen et al. 2005) and the assessment of basic synaptic mechanisms is restricted to systemic administration of broadly acting neuromodulatory drugs in healthy subjects (Donchin et al. 2002). More invasive methods are achievable in non-human primates (Scott et al. 2001; Prsa et al. 2010, Paz et al. 2005, Li et al. 2001). However, these studies are limited by small experimental sample sizes and the large effort required to train the animal and address ethical concerns.

Here, we introduce the ETH Pattus, a novel robotic platform designed for motor learning experiments in rodents. This device allows us to quantitatively assess forepaw movement kinematics and dynamics and to implement force fields to guide or perturb the motion in a well-controlled and repeatable manner. The possibility to have an automated experimental setup, task evaluation and rewarding system not only increases the time efficiency, but the exclusion of the human operator as a potential source of experimental error also constitutes a substantial improvement.

Several robotic devices designed for interaction with rats have been reported in literature, demonstrating the successful integration of robotic equipment with such animal models. While most of the previous work has focused on the development of robotic systems to assess and train locomotor functions of spinal cord injured (SCI) rodents (de Leon et al. 2002, Nessler et al. 2005, Udoekwere et al. 2006), devices for interaction with the rat forelimb have also been reported. Fowler et al. used a basic force-sensing operandum to investigate the effect of a specific drug on the continuous pressure exerted by the rat (Fowler et al. 1994). Further, a one degree-of-freedom (DOF) lever arm was designed to investigate feedforward and feedback control mechanisms (Francis and Chapin 2004). However, despite their usefulness in particular experiments, these simple mechanisms have limitations when studying more complex motor learning paradigms.

In this paper we present the design, implementation and evaluation of the ETH Pattus, a robotic platform to investigate motor learning during rat forelimb movements. We report preliminary data of healthy animals collected under four experimental paradigms aimed at (i) validating the usability of the ETH Pattus with rats, and (ii) demonstrating the possibility of influencing and quantitatively evaluating forepaw kinematics and dynamics. We hypothesized that rats could get familiarized with the robotic device, and that their motor behaviour could be shaped through the application of force fields generated by the robot. Furthermore, potential avenues for future studies are discussed.

2. The ETH Pattus

2.1. Requirements

Using a robotic device that the rat actively manipulates in a certain way to obtain a food reward can allow the study of explicit forelimb movements. A typical task would require the rat to grasp a handle end-effector and perform a very specific movement, e.g. pull the handle over a predefined distance under various loading conditions. Several aspects were taken into consideration during the design of our system, so that the robotic manipulandum would match the kinematics and dynamics of the rat forelimb:

- *Degrees of freedom* – this criterion is defined by the complexity of the task we wish to attain during motor learning experiments. The robotic device should allow in-plane movements for investigations of reaching tasks and planar force field adaptation, but also pronation/supination of the rat forelimb. The latter DOF is particularly important for studying learning of forelimb reaching skills (Whishaw et al. 1991), e.g. grasping a food pellet and moving it to the mouth.
- *Rat forearm kinematics* – the workspace of the device should match the range of motion of the rat forearm in the sagittal plane. The required workspace for the rat manipulandum was estimated to be 40x20mm^2 based on studies on rat locomotion in terms of paw and limb length, as well as joint angles (Fisher et al. 2002, Thota et al. 2005, Bennett 2009). Furthermore, the shape and size of the end-effector must accommodate for a firm grasp of the animal. A sphere matching the dimensions of the rat paw was found likely to be the object shape a rat could grasp the easiest, and further resembles the food pellets typically used in such studies.
- *Rat forearm dynamics* – maximal joint angles and torques in rodent models during locomotion were reported in (Bennett 2009). A maximum force of 2.3N exerted by the rat at the paw level was thus estimated by combining the length of the forearm with the maximum joint moments. No scientific literature was found reporting the maximum pronation and supination torques of the rat forelimb. These values were determined based on the study of O'Sullivan and Gallwey which examined pronation and supination torques in human male subjects (O'Sullivan and Gallwey 2002), and downscaled with respect to the mass of the rat, assuming a proportional relationship between mass and achievable torque according to the laws of similitude. This resulted in a 49mNm maximum pronation and 61mNm maximum supination torques estimation. The robotic device should be able to render dynamic environments by

implementing various force fields to challenge the rat during the motor learning paradigms. The fast interactions with the rat forelimb require high dynamics and transparency on the side of the robot, translating into a relatively high mechanical stiffness, high velocity, low friction and low inertia at the level of the end-effector. Additionally, for a proper rat-robot interaction, smooth control of the device with a sufficiently high update rate is required.

- *Integration with the training environment* – current rat training environments are operated manually (Schubring-Giese et al. 2007) or include training cages with automated doors, sound systems and food pellet dispensers. These elements should be modularly integrated with the new robotic platform, providing an automated system for motor learning experiments. Safety is also a major concern in experimental studies involving animals, hence mechanical, electrical and software emergency units require particular attention as well.

2.2. Design and implementation

Considering the rapid movements of the rat forelimb and the need for a low inertia device, a parallel mechanical structure was chosen for the design of the ETH Pattus. Parallel robots offer high structural stiffness within light constructions thanks to the grounded actuators, while enabling high accelerations and very small positioning errors at the level of the end-effector (Clavel 1988). The parallel mechanism on which we based our design is the Pantograph, a 2 DOF, five-bar-linkage planar mechanism (Campion et al. 2005). Although this can provide adequate complexity for training and measuring precision forelimb movements in rats, several mechanical changes had to be performed in order to adapt the workspace and downscale the output forces to match those of the rat forepaw kinematics and dynamics. In addition, an extra rotational DOF has been implemented to allow forelimb pronation/supination. Three electromagnetic motors coupled with high-resolution optical encoders provide the required actuation and position measurement, as well as a good velocity estimate of the device end-effector. Control is performed via a multirate timed-loop structure, with the main low level impedance control running at 200*Hz* and two additional loops, one for visualizing and recording images of the rat-robot interaction with a commercial web camera (Logitech HD Webcam C270) running at 30*Hz*, and the other for recording of all experimental data (e.g. end-effector positions, velocities, motor torques, etc.) running at 1*kHz*. This high sampling rate is motivated by the fast movements of rodents, who are able to accomplish a reaching task within ~200*ms*. The mechanical configuration and the main components of the ETH Pattus is depicted in Figure 1, while a detailed description of the design, robot kinematics, singularities, mechanical constraints and electronics can be found in (Vigaru et al. 2011).

2.3. Evaluation

2.3.1. General features

The ETH Pattus has a compact structure, with external dimensions of 150x235x230mm^3. Using the kinematics dimensions and equations, the workspace of the manipulandum was calculated and overlaid with the rat forelimb kinematics to determine an area of approximately 50x30mm^2. The position bandwidth of the device was previously identified to be 15*Hz*, with a resonance frequency of the system around 11*Hz* (Vigaru et al. 2011). This rather low value for a parallel structure is explained by friction and play in the telescopic shaft; nevertheless it is sufficient for interaction with rat movements during our experiments.

2.3.2. Friction identification

Friction influences the dynamics and sensitivity of the manipulandum, as well as the quality of interaction with the rat. Static and dynamic friction has been experimentally identified in the *x*- and *y*-directions throughout the planar workspace and is presented in Figure 2. Static friction was determined by increasing the motor current in small increments until movement of the end-effector was detected. The dynamic friction was measured by moving the end-effector at constant velocities and recording the current (torque) supplied to the actuators. The measurement variability is mainly due to the mechanical play in the telescopic shaft.

2.3.3. Output force

The force manipulability ellipsoid (Fig. 3, top) was first computed using the eigenvalues and eigenvectors of JJ^T, where J represents the Jacobian of the kinematic structure, obtained by differentiating the forward kinematics map (Vigaru et al. 2011) with respect to the actuated joints:

$$J = \begin{bmatrix} J_{11} & J_{12} \\ J_{21} & J_{22} \end{bmatrix} \quad (1)$$

where:

$$J_{11} = \frac{\partial x_h}{\partial \theta_1} + \frac{h}{d}\left(\frac{\partial y_4}{\partial \theta_1} - \frac{\partial y_2}{\partial \theta_1}\right) - \left(\frac{\partial h}{\partial \theta_1} d - \frac{\partial d}{\partial \theta_1} h\right)\frac{y_4 - y_2}{d^2} \quad (2)$$

$$J_{12} = \frac{\partial x_h}{\partial \theta_2} + \frac{h}{d}\left(\frac{\partial y_4}{\partial \theta_2} - \frac{\partial y_2}{\partial \theta_2}\right) - \left(\frac{\partial h}{\partial \theta_2} d - \frac{\partial d}{\partial \theta_2} h\right)\frac{y_4 - y_2}{d^2} \quad (3)$$

$$J_{21} = \frac{\partial y_h}{\partial \theta_1} - \frac{h}{d}\left(\frac{\partial x_4}{\partial \theta_1} - \frac{\partial x_2}{\partial \theta_1}\right) - \left(\frac{\partial h}{\partial \theta_1} d - \frac{\partial d}{\partial \theta_1} h\right)\frac{x_4 - x_2}{d^2} \quad (4)$$

$$J_{22} = \frac{\partial y_h}{\partial \theta_2} - \frac{h}{d}\left(\frac{\partial x_4}{\partial \theta_2} - \frac{\partial x_2}{\partial \theta_2}\right) - \left(\frac{\partial h}{\partial \theta_2} d - \frac{\partial d}{\partial \theta_2} h\right)\frac{x_4 - x_2}{d^2} \quad (5)$$

with $h = \overline{P_3P_h}$ and $d = \overline{P_2P_4}$ (cf. Fig. 1, left).

Due to the orthogonality between velocity and force manipulability, the largest forces can be applied in the directions where the maximum velocity is the lowest. The simulations were verified by measuring the real output force of the robot using a commercial force sensor (Nano17; ATI Industrial Automation, USA) attached to the sphere end-effector. The device is shown to produce an output force of at least 2.11 N throughout the reachable workspace (Fig. 3, bottom), which is in accordance with the simulated values.

2.3.4. Safety

In order to prevent injury of the rat or damage of the robot, several safety elements have been implemented such as damping at high velocity above an adjustable threshold, motor current limitations and mechanical workspace constraints. Additionally, an emergency switch integrated into an operator console is available at all times for the human observer to stop the system, should an unexpected event occur. Two additional push-buttons are incorporated in the operator console, offering the possibility to manually override the automated performance rating during the trials.

The key characteristics of the ETH Pattus are summarized in Table 1.

3. Experimental validation

3.1. Animals

Ten adult male Long-Evans rats (14 weeks old, 220-240*g*) were trained on the ETH Pattus. Animals were separated into two experimental groups, composed of 6 animals and 4 animals respectively, which were trained according to different experimental paradigms. Animals were housed in groups of two per cage on a 12*h*/12*h* light/dark cycle. Training sessions were performed during the dark phase. Animals were food-deprived for 24*h* before the first training session. Subsequently, daily food supplements (40-60*g/kg* of standard diet adjusted to maintain constant body weight) were given after training. Access to water was ad libitum.

3.2. Training environment

A detailed description of the experimental setup is presented in Figure 4. A custom-made Plexiglas chamber with a vertical window (width: 1*cm*, height: 5*cm*) in the front wall was used. The window was always open. A tray was mounted in the rear part of the chamber, which was served with food pellets (45*mg*, Bioserve Inc., Frenchtown, NJ) by a pellet dispenser (Lafayette Instrument Comp., USA, Model 80208). When a reward constraint was fulfilled, an auditory cue (beep sound, 1*sec*) was presented to the rat, while a pellet was released to the tray. The robotic device was attached to the cage such that the end-effector was centered in front of the window. In the present study the rats used the preferred limb to perform the movements, but this could also be constrained with a Plexiglass box positioned inside the cage in a way to limit window access to the desired limb (Fig. 4).

3.3. Experimental procedures

The objective of the initial experiments performed with healthy rats using the ETH Pattus was to investigate two key hypotheses crucial for future motor learning experiments in rats, being:

(i) can healthy rats become familiar with the experimental environment and grasp the end-effector of the robotic manipulandum, as measured by an increasing number of successful touches and (ii) can force fields implemented on the robotic device perturb or shape forepaw kinematics and kinetics during a pulling movement. Four experiments were implemented in order to answer these questions. Experiment 1, consisting of pre-training, was performed by all animals. The first group of 6 animals (4 right handed rats) successively performed experiments 2, 3 and 4. The second group of 4 animals (all right handed) alternated between experiments 2 and 3.

3.3.1. Experiment 1: Touching the end-effector

Prior to training with the robot, animals were acclimated to the training cage and the food pellets for 1*h* (pellets were mixed with conventional food in a tray inside the cage). On the subsequent day the end-effector of the manipulandum was presented at 4*mm* distance to the window. Behavior was initially shaped by manually given rewards by the investigator whenever the rat approached the window or tried to touch the manipulandum. Further motivation was given by presenting a food pellet close to the end-effector. The robot triggered a reward if an end-effector displacement of 0.2*mm* in either *x*- or *y*-directions occurred within a time window of 180s upon presentation of the end-effector; the time window was retained for all 4 experiments. Support from the investigator was progressively reduced and ended after the rat touched the end-effector 10 times. Pre-training (operant conditioning/instrumental learning) was continued until the animal performed 100 trials during 45 minutes over two subsequent sessions. Furthermore, throughout all of the experiments, when a trial was either successfully completed or failed by the rat, the robot was retracted to an initial position away from the reachable workspace of the rat, before a new trial was initiated.

Experiment 1 was performed to familiarize the rat to the cage and to the experimental setup and to produce a frequent, repetitive interaction that allows for future experiments. All rats needed to accomplish this task, though we show here only the data from the 6 animals of the first experimental group.

3.3.2. Experiment 2: Pulling 10mm in a null field

For this experiment, the start position of the manipulandum was shifted on the *y*-axis to $18mm$ distance to the cage wall, centered in *x*-direction in front of the window. Reward criteria were a continuous pull of the manipulandum over $10mm$ in the *y*-direction (without any stops along the way), and reaching a target area in front of the window, invisible to both the rat and the investigator (10x8mm). The animals thus needed to reach out of the window, grasp the manipulandum and pull it towards the cage. A session was ended after 100 trials or 45 minutes.

The goal of this experiment was to establish a more complex form of rat-robot interaction, similar to single pellet reaching tasks (Whishaw and Pellis 1990, Buitrago et al. 2004).

3.3.3 Experiment 3: Pulling 10mm in a haptic tunnel

For this experiment, the start position of the manipulandum remained the same as in experiment 2, but now a haptic tunnel was rendered by the robotic device to restrict the lateral movement outside the range of a predetermined deadband (i.e. the tunnel), thus guiding/assisting the rat to perform a longitudinal movement along a straight path. The haptic tunnel was implemented as a stiff spring-damper component to limit the lateral displacement of the end-effector, producing a correction force when a deviation Δx from the tunnel wall was detected (Eq. 6). Spring constant $k = 0.75N/mm$ and damping constant $b = 0.0002N \cdot sec/mm$ were empirically determined to achieve stable interaction.

$$F_x = \begin{cases} k \cdot \Delta x + b \cdot \dot{x}, \ if \ |x| > |x_w| \\ 0, \qquad otherwise \end{cases} \tag{6}$$

where $\pm x_w$ represent the wall positions of the tunnel.

Reward criteria were a continuous pull of the manipulandum over $10mm$ in *y*-direction and reaching a target area as described in experiment 2.

The motivation for this experiment was to evaluate the effect a guiding force field could have on the endpoint trajectory, and to establish whether the force generated by the ETH Pattus is suitable to shape the movement of the rat.

3.3.4. Experiment 4: Pulling 10mm in a velocity-dependent force field

The start position was at y=18*mm* as in experiments 2 and 3. Reward criteria were a continuous pull of the manipulandum over 10*mm* in the *y*-direction and reaching a target area as described in experiments 2 and 3. In this setup a velocity-dependent force field (negative damping) along the *x*-direction (either left or right, depending on the handedness of the rat) proportional to the velocity of the end-effector in the *y*-direction (reaching) was implemented:

$$\begin{pmatrix} F_x \\ F_y \end{pmatrix} = \underbrace{\begin{pmatrix} 0 & b \\ 0 & 0 \end{pmatrix}}_{B} \cdot \begin{pmatrix} \dot{x} \\ \dot{y} \end{pmatrix} \tag{7}$$

where F_x and F_y are the forces applied by the robot in the two directions, $\dot{x}$ and $\dot{y}$ represent the endpoint velocities, while b is a constant comprising the damping of the induced environment in endpoint coordinates. For a specific force field to be produced at the end-effector level, position and velocity information at the two actuated base joints is required. In this way endpoint force fields can be translated into torques to be applied by the two motors. The corresponding motor torques are calculated from Equation (3):

$$\tau_M = J^T \cdot B \cdot J \cdot \dot{\theta} \tag{8}$$

where τ_M represents the motor torques vector, J is the Jacobian derived from the robot kinematics and $\dot{\theta}$ embodies the angular velocity vector of the two motors controlling the *x-y* planar movement.

The goal of this experiment was to evaluate the effect of a perpendicular force field applied to the manipulandum during pulling movements. This type of force field is suitable to investigate motor adaptation, and has found wide application in human studies (Shadmehr and Mussa-Ivaldi 1994, Scheidt et al. 2000, Franklin et al. 2008).

3.4. Data analysis

Joint positions and velocities as well as motor currents were recorded at a sampling frequency of 1*kHz* and stored for offline analysis. Robot data were processed using MATLAB R2010a (The MathWorks, Inc.). To investigate the ability of the rats to perform the respective tasks during each experiment, parameters were extracted from the kinematic data collected by the robot during each trial. In experiment 1, the percentage of touches of the end-effector achieved below a time threshold of 20 seconds p_T during an entire session was calculated as indicator of how successful the rat was at achieving the task. Given that the repositioning phase of the robot required 10 seconds to retract and return to the start position between trials, this time threshold allows 10 seconds for the rat to touch the robot after the end-effector is positioned in front of the cage. To evaluate rat movements and quantify the influence of force fields during experiments 2 to 4, the integrated error ε to a virtual straight-line from the start position, normalized over the duration of the movement, was computed for each successful movement, i.e. movements reaching the target area within a time window of 180 seconds. For each parameter, a repeated measures ANOVA was performed to test for statistically significant differences in between experimental sessions. The level of significance was set to 0.05.

4. Results

A progressive and significant increase in p_T was observed over the consecutive sessions of experiment 1. Figure 5 shows the evolution of the mean p_T for the first group of 6 animals that performed 4 sessions of experiment 1. An average p_T of 87.7% was observed in the last session of pre-training, while this number was only 20.6% in the first session ($p<0.001$). This illustrates that operant conditioning was successful, and that it was possible to train rats to interact with the device within 3 experimental sessions (i.e. less than 3 hours).

Figure 6A presents average trajectories of successful trials over sessions of experiments 2, 3 and 4, where the 6 rats of the first experimental group performed 10*mm* pulling movements in a null field (⃠), a haptic tunnel (||) and a velocity-dependent force field (VF), respectively.

Shaded areas represent 95% confidence intervals. In the null field condition, characteristic ("natural") pulling movements for each rat can be observed, as the device did not render any force field and only the small output impedance of the ETH Pattus was felt by the animal. Note that R2 and R6 performed the task with their left forepaw, while the other rats used their right forepaw. With the haptic tunnel, movements in *x*-direction were limited by the virtual wall, thus resulting in much straighter and less variable trajectories. Finally, in the presence of the velocity-dependent force field, the trajectory of the pulling movement became curved. The endpoint movement was clearly shifted to the side of the target area in all rats. Figure 6B illustrates an example of kinetic and dynamic data captured by the robot, and presents the mean velocity profiles along the *x*-axis (i.e. in which the force is applied) for two sessions of one representative animal (R2), in the null field and in the velocity-dependent force field. In the null field, the velocity in the *x*-direction remained small due to the non-straight natural trajectory. In the case of the velocity-dependent force field, although the first part of the movement presented a similar velocity profile, the effect of the force field could clearly be identified by the presence of a velocity peak. Another example of dynamic data the robot can record is presented in Figure 6C, which shows the average interaction force applied against the haptic tunnel (i.e. in the *x*-direction) during pulling movements for rat R2. This interaction force with the haptic tunnel can be explained by the fact that "natural" pulling trajectories do not follow a straight line and that rats would initially tend to deviate and penetrate into the haptic tunnel. It can be observed that the interaction force tended to decrease over training sessions in the haptic tunnel.

The influence of the haptic tunnel on movement behavior is further quantified in Figure 7 by presenting the average integrated error ε per session for the second group of 4 animals who performed a succession of training sessions in the null filed (⃠) and the haptic tunnel (||). The initial session in the null field showed an average integrated error ε of 21.2mm^2 caused by the curvature of the "natural" pulling movement of the rats. In the sessions with the haptic tunnel a strongly significant reduction in ε to 4.3mm^2 is observed (-79.7%, $p<0.01$), as the deviation in *x*-direction was limited to ±0.5*mm*. After three consecutive training sessions in the haptic tunnel, the force field was removed and animals were tested for two sessions in the null field. Interestingly, in the session directly following training in the haptic tunnel, the mean ε remained significantly smaller than in the initial session (15.0 mm^2, $p<0.001$). This suggests that the motor behavior of the rats during pulling movements was shaped by the force field, leading to a straighter pulling movement even after the haptic tunnel was removed. This effect was only temporary and washed out in the following session, resulting in an increased ε similar to that of the first session (19.4 mm^2).

5. Discussion and outlook

This paper presents and experimentally validates the ETH Pattus, a 3 DOF small-scale robotic manipulandum consisting of a Pantograph structure with an additional rotational DOF, to investigate planar reaching and pronosupination movements, such as required when grasping a food pellet and moving it to the mouth. Such skilled reaching tasks represent frequently used skill learning paradigms for rodents (Whishaw and Pellis 1990, Metz and Whishaw 2000) that allow the examination of movement kinematics in order to monitor the improvement of motor performance within and between training sessions and identification of fast and slow components of motor learning (Buitrago et al. 2004). The ETH Pattus enables the automation of time-consuming training periods and offers the possibility to quantitatively assess endpoint kinematics and kinetics of rat movement.

Performance evaluation showed that the device has a sufficiently large workspace to match the kinematics of the rat's forelimb. From the dynamics point of view the robot exhibits low friction (<50 mN), while the output force (>2 N) enables rendering of virtual dynamics in a controlled and repeatable manner, allowing the implementation of various force fields that can significantly influence rat's forelimb movements. These are unique features compared to existing robotic devices design to interact with rat forelimbs (Fowler et al. 1994, Francis and Chapin 2004).

Preliminary experiments with healthy rats provided evidence for the ease of use of the ETH Pattus and the possibility for animals to learn how to touch and interact with the robot in less than 3 one-hour sessions. Kinematic and dynamic data collected during interaction within different training conditions suggest that rats modify their motor behavior in response to force fields. Variations in the velocity profiles indicate that rats were not simply being pushed away by force fields, but had some control over the pulling movements. Similar to humans (Morasso 1981, Flash and Hogan 1985, Bullock and Grossberg 1988, Gordon et al. 1994, Harris and Wolpert 1998, Moran and Schwartz 1999), velocity profiles in rat movements exhibited bell-shaped profiles, which could suggest comparable motor control and optimization strategies. An inter-session decrease in interaction force within a haptic tunnel force field illustrates that rats progressively reduced contact with the virtual wall by producing trajectories that were closer to the straight line. These results were in accordance with the straighter trajectories produced after removing the haptic tunnel. While representing promising results similar to what has been observed in studies on human and non-human primates using robotic devices (Shadmehr and Mussa-Ivaldi 1994, Scheidt et al. 2000, Franklin et al. 2008, Reinkensmeyer and Patton 2009, Huang and Krakauer 2009, Scott 1999, Li et al. 2001), these observations should be interpreted with care, given the high variability in interaction force and the limited number of animals and training sessions.

Apart from investigating motor skill learning and motor adaptation, this robotic platform may become a valuable tool to assess recovery after brain lesions such as ischemic stroke. Again, the application of a conventional skilled reaching task before and after an experimentally induced stroke (Schubring-Giese et al. 2007) is limited by the lack of an objective and precise quantification of movement sequences. Here, measurement of endpoint kinematics and dynamics could reveal even subtle changes in movement patterns between the pre- and post-lesional condition. Furthermore, such measurements would also be a prerequisite for the precise quantification of functional effects of therapeutic interventions like rehabilitative training (Biernaskie et al. 2004) or the application of drugs (Goldstein 2000) in rodent models. Finally, this approach could be used to evaluate the therapeutic effect of different modes of robotic assistance on large and homogeneous populations of animal models, such as error amplification, which has been tested in stroke patients (Patton et al. 2006).

From the mechanical point of view, important directions for future improvements of the ETH Pattus include the development of an orientation mechanism for studying movements in different planes, as well as the addition of a force sensor in the end-effector to allow for grasping force measurement, improve touch detection and quantify interaction force directly at the level of the end-effector. Furthermore, an evaluation of the pronation/supination DOF is yet to be performed, which will further allow the implementation of more complex motor skill reaching tasks.

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Figure Captions:

Figure 1. Mechanical structure of the ETH Pattus; left: CAD rendering - top view; right: 3D view showing the main components.

Figure 2. Static and dynamic friction forces measured in the *x*- (top) and *y*- (bottom) directions, with 2nd order polynomial fits and their respective correlation coefficients.

Figure 3. Maximum force exerted at the end-effector; top: simulation of force manipulability ellipsoid; the white rectangle represents the reachable planar workspace; bottom: measured output force (mean and standard deviation over 4 measurements) at 6 different points spanning the reachable workspace.

Figure 4. System diagram (left) and experimental setup (right) showing the interconnection between the various elements and the interaction between the rat and the robotic manipulandum.

Figure 5. Mean ± standardized error of the mean (SEM) of the percentage of trials performed in less than 20 seconds p_T for the 6 rats that performed 3 sessions of experiment 1 (S1-S3). ** indicates p<0.01, *** indicates p<0.001.

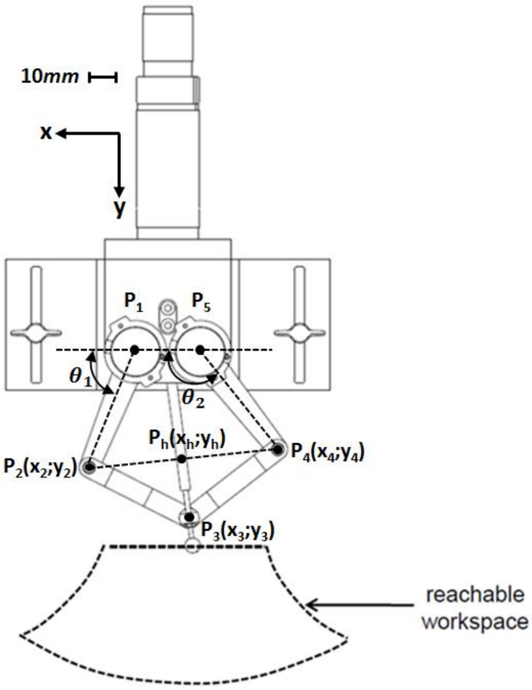
Figure 6. Forepaw kinematic and dynamic data during pulling movements performed with the robotic device. A: Mean *x-y* trajectories of pulling movements during one session (100 trials) of each force field (null field ⃠, haptic tunnel ||, and velocity-dependent force field VF) for each animal (R1-R6). Shaded areas represent 95% confidence intervals. Grey boxes represent the target area to reach for a trial to be rewarded. B: Mean velocity profiles along the *x*-axis during pulling movements for one session in the null field, and one session in the velocity-dependent force field for one representative animal (R2). For comparison purpose, plots are normalized over the trial duration. C: Mean profiles of interaction force in the *x*-axis for two consecutive sessions of pulling movements with the haptic tunnel for one representative animal (R2).

Figure 7. Mean ± standardized error of the mean (SEM) of the integrated error with respect to the straight line for 4 animals during consecutive sessions in the null field (⃠) and the haptic tunnel (||). **: p<0.01, ***: p<0.001.

Table Captions:

Table 1. Characteristics of the rat manipulandum.

Figure 1.


10mm
x
y
P1
P5
θ1
θ2
Ph(xh;yh)
P2(x2;y2)
P4(x4;y4)
P3(x3;y3)
reachable
workspace

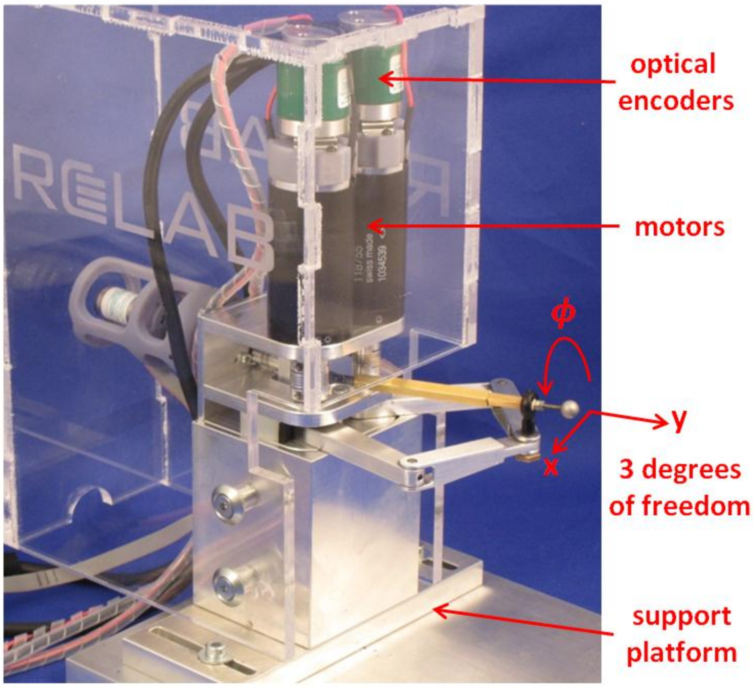

optical
encoders
motors
ϕ
y
x
3 degrees
of freedom
support
platform

Figure 2.

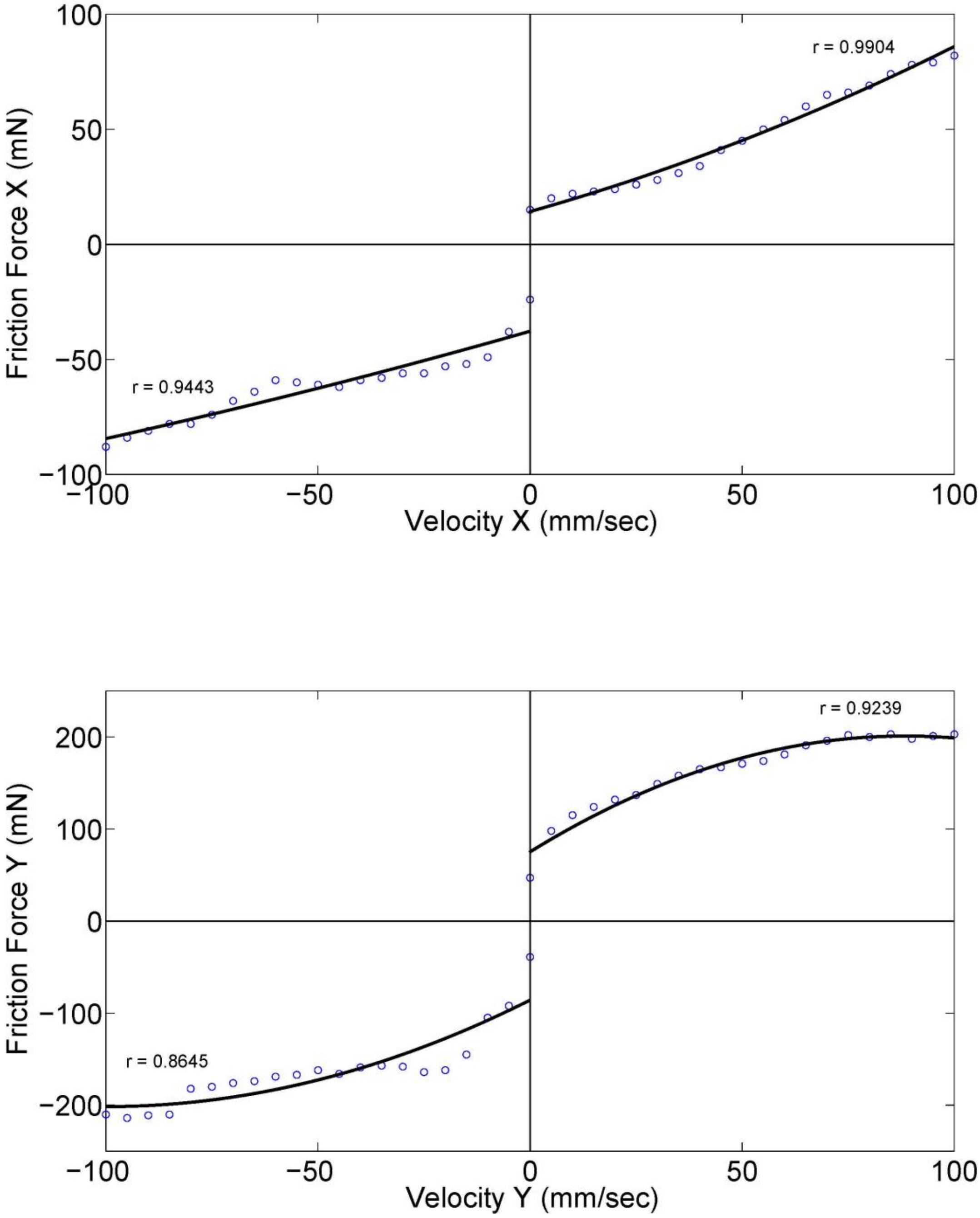

r = 0.9904
r = 0.9443
Friction Force X (mN)
Velocity X (mm/sec)
r = 0.9239
r = 0.8645
Friction Force Y (mN)
Velocity Y (mm/sec)

Figure 3.

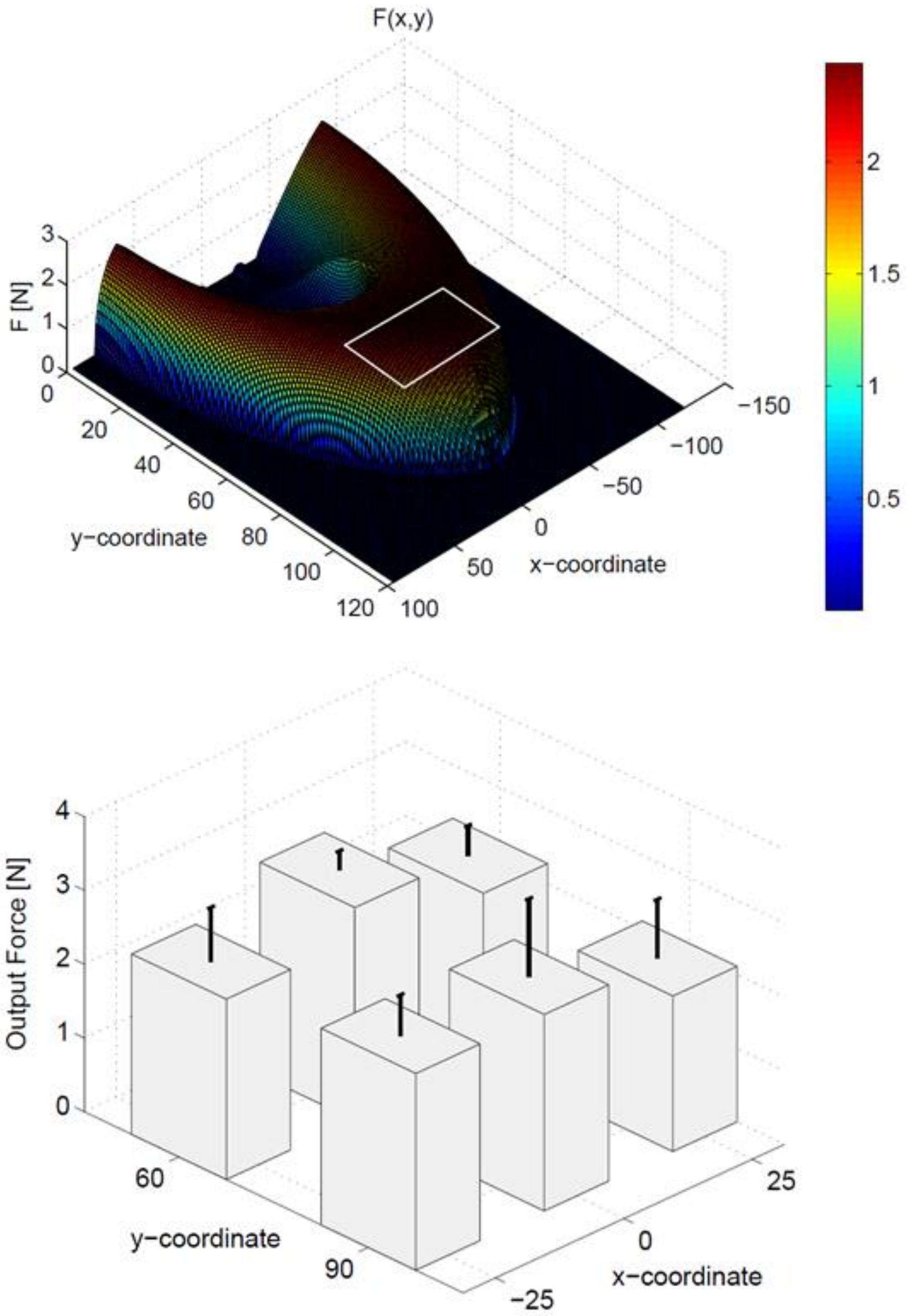

F(x,y)
F [N]
y−coordinate
x−coordinate
Output Force [N]
y−coordinate
x−coordinate

Figure 4.

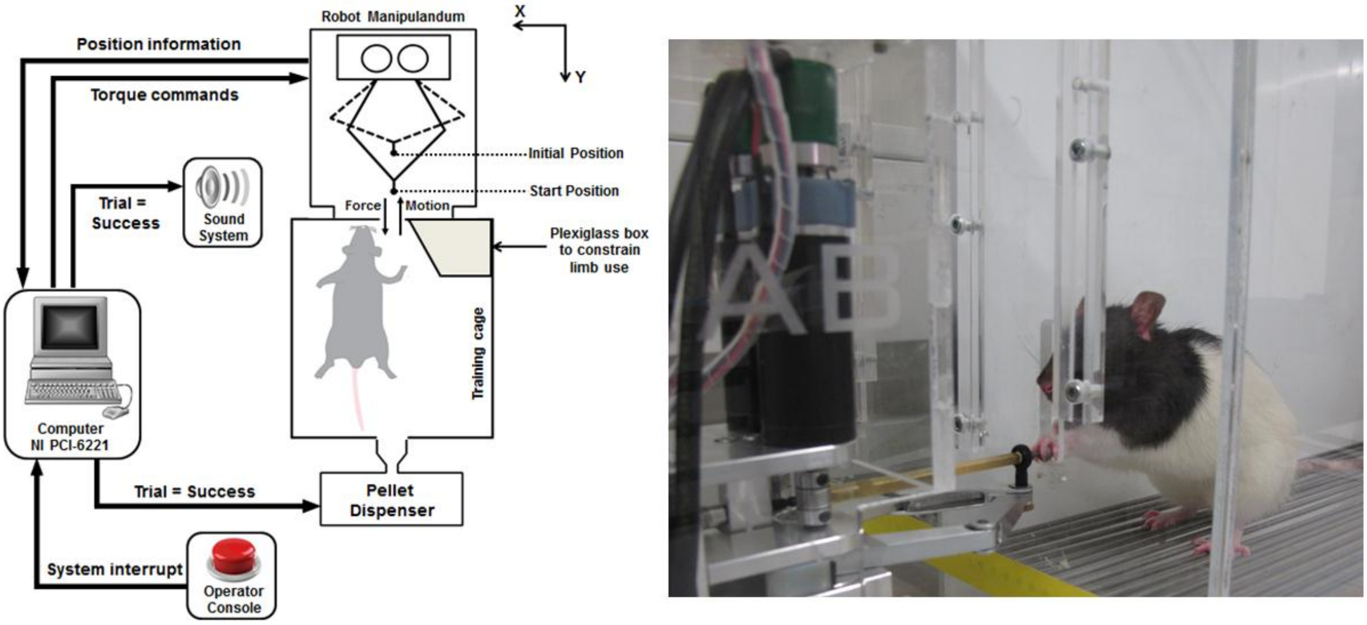
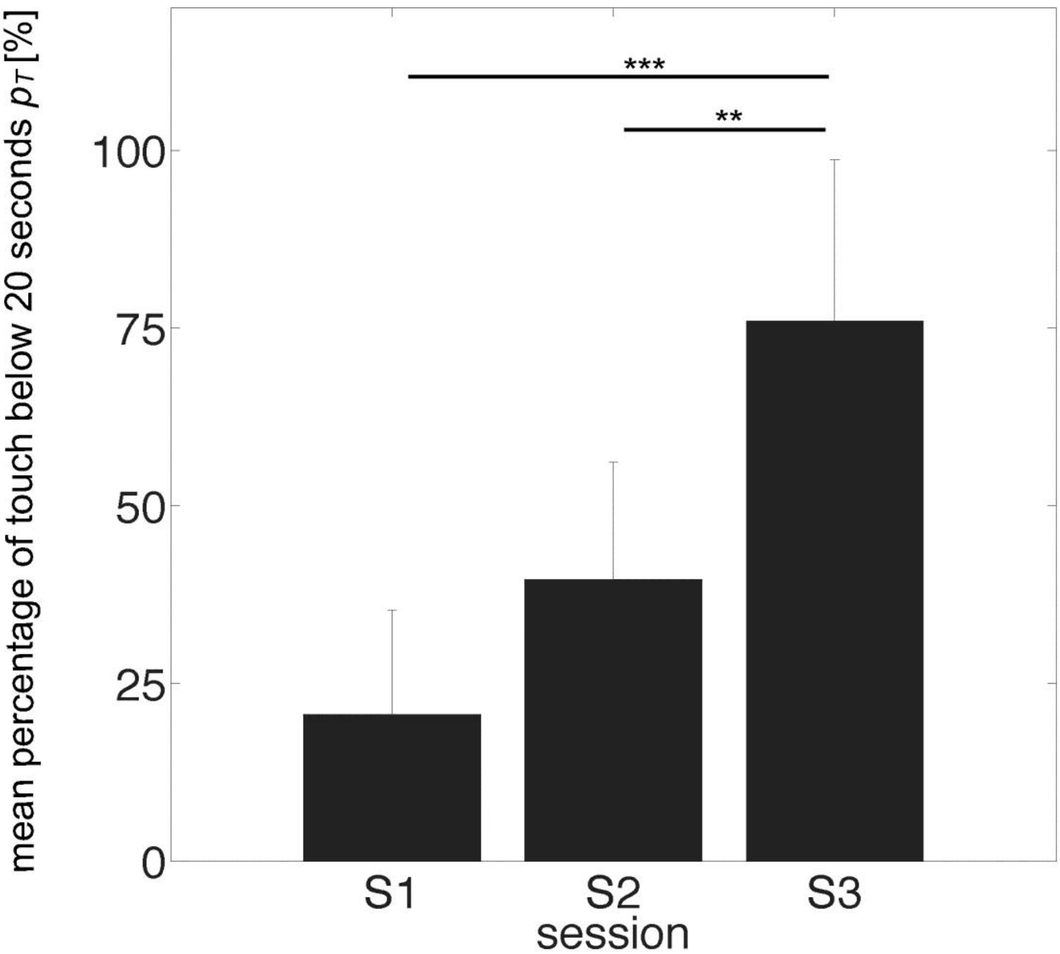
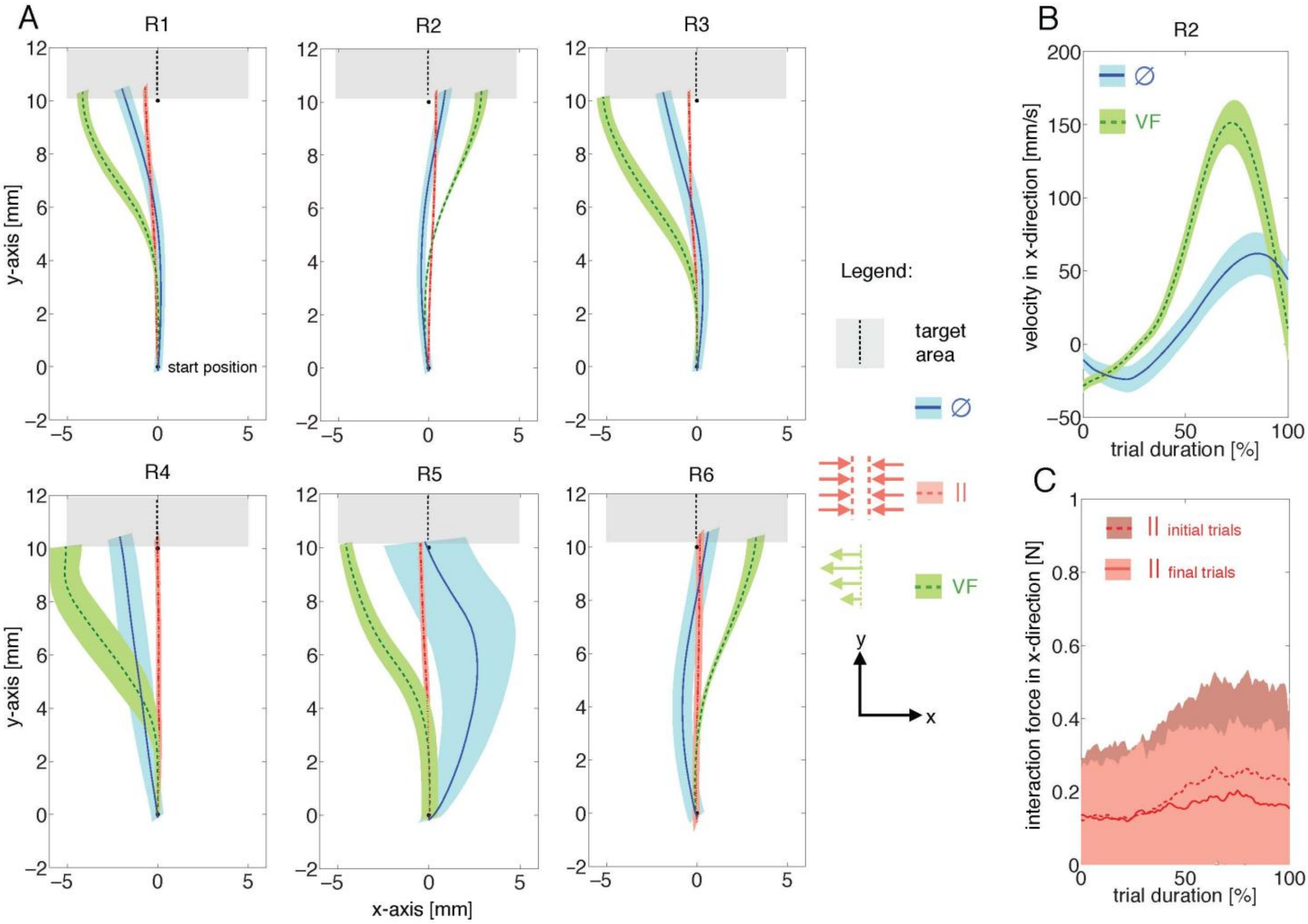

Robot Manipulandum
X
Y
Position information
Torque commands
Initial Position
Start Position
Trial =
Success
Sound
System
Force
Motion
Plexiglass box
to constrain
limb use
Training cage
Computer
NI PCI-6221
Trial = Success
Pellet
Dispenser
System interrupt
Operator
Console

Figure 5.



mean percentage of touch below 20 seconds p_T [%]
***
**
100
75
50
25
0
S1
S2
S3
session

Figure 6.



A
R1
R2
R3
R4
R5
R6
y-axis [mm]
x-axis [mm]
start position
Legend:
target area
∅
||
VF
y
x
B
R2
velocity in x-direction [mm/s]
trial duration [%]
C
|| initial trials
|| final trials
interaction force in x-direction [N]
trial duration [%]

Figure 7.

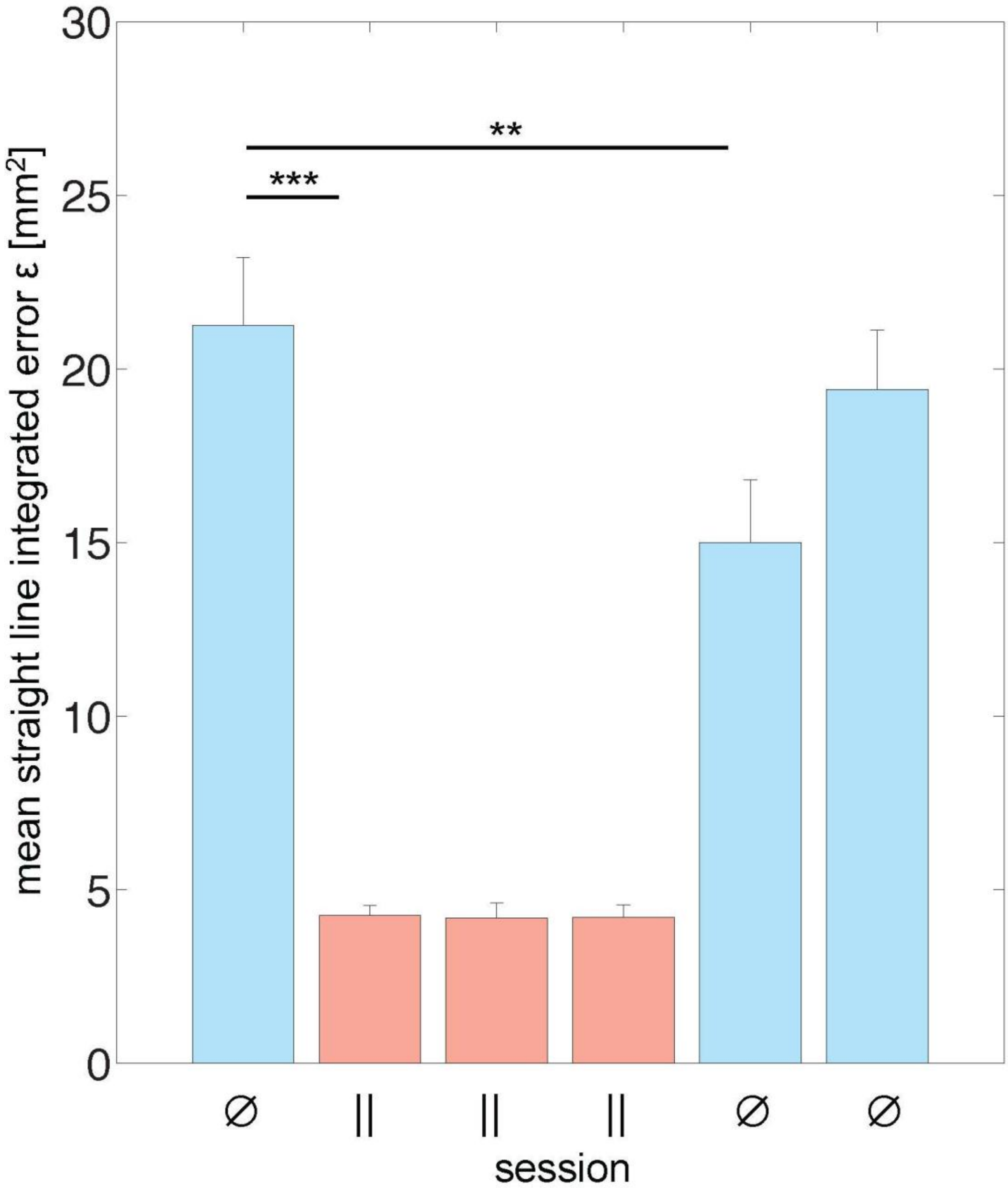

30
25
20
15
10
5
0
mean straight line integrated error ε [mm2]
**
***
∅
||
||
||
∅
∅
session

Table 1.

external dimensions	150x235x230 *mm*^{3}
reachable planar workspace	50x30 *mm*^{2}
position resolution (end-effector)	0.015 *mm* / *encoder count*
velocity resolution (end-effector) @ 200 *Hz*	3 *mm / s*
maximum continuous output force (end-effector)	2.11 *N*
pronation / supination torque	126 *mNm*
static friction (*x- / y*-direction)	19.6 / 43.1 *mN*
sphere end-effector diameter	6 *mm*
closed-loop position bandwidth	15 *Hz*
controller frequency	200 *Hz*

The following paragraphs represent the responses to the reviewers of our September 2011 submission of this manuscript for the Special Issue on Motor Learning and Neuro-Rehabilitation of the IEEE Transactions on Neural Systems and Rehabilitation Engineering.

We would like to thank the reviewers for their thorough review and the valuable comments. In the following, we first describe the main changes that have been made to the manuscript, and then address the remaining issues point-by-point (in Italic in the text).

Main changes to the manuscript:

1. *The paper has been restructured in order to give more weight to the preliminary results of the animal studies, including a better motivation for the experimental protocol (section 3: Experimental validation).*
2. *Further experimental data have been collected with 6 new rats, following a more standardized protocol.*
   We now present (section 4: Results) group results and kinetic data.
3. *The introduction and discussion sections have been rewritten, to more strongly underline our hypothesis, results and future research direction with respect to motor learning investigations.*
4. *The manuscript has been proof-read by a native English speaker.*

Point-by-point answers to reviewers:

Reviewer: 1

It seems there is a substantial overlap between the present manuscript and a prior published full article from the authors.
Vigaru, B.; Lambercy, O.; Graber, L.; Fluit, R.; Wespe, P.; Schubring-Giese, M.; Luft, A.; Gassert, R.; A Small-Scale Robotic Manipulandum for Motor Training in Stroke Rats, 2011 IEEE International Conference on Rehabilitation Robotics (ICORR), June 29 2011-July 1, Zurich 2011
This study was designed to investigate motor learning and recovery in rats with a new robotic system for forelimb. It describes the mechanical structure, electronics and control. Results showed rats can grasp the end-effector of the robotic manipulandum. The force field can be implemented for haptic guidance and perturbation. It is better to revise the manuscript including only those unique data to minimize the overlap in publication. Since the prior publication already include the detail in the mechanical structure and Actuation, Sensors and Control.

The paper has been carefully revised, restructured and the description of the robotic device has been shortened to minimize information overlap. Whenever technical details presented in the ICORR paper are not crucial to the understanding of the present manuscript, these have been omitted. The ICORR paper is clearly referenced in the text. The sections on the mechanical structure and electronics (section 2.2 in the new document) have been shortened. The previous Table 1 (requirements) has been removed, and changes have been made to figures 1 and 4. Furthermore, we believe that the experimental validation and preliminary results with rats (sections 3 and 4) presented in the revised version of this paper considerably extend the content of the manuscript, and make it go well beyond what was reported in the ICORR paper (concept, mechanical design, kinematics and control).The implementation of force fields is also novel in the present manuscript.

Specific comments:

1. Figure 7 shows 3 representative trajectories, it is better to provide the results from all the four rats, similar to the figures 5, 6 and 8.

Figure 7 has been replaced with new one (figure 6 in the new document) showing representative trajectories from 6 animals (new experiments with 6 rats were performed after the previous submission). Figure 6 now presents mean trajectories with 95% confidence intervals over entire training sessions for each of the force fields, for the 6 rats. This illustrates good intra-session repeatability of the trajectories for each rat, and the different force fields influence the trajectories of all rats in a similar way.

2. 8 Figures and 2 tables in the manuscript, it is better to focus the paper in the key findings and reduce the number of figures.

Figures 5-7 replace the old figures 5-8, in order to better explain the key findings. Table 1 has been removed as well. The paper now contains 7 figures and 1 table, which we believe are necessary for the reader to gain an optimal understanding of the paper.

3. Although the system has been tested on four rats, if this robotic system is designed for stroke rats, then how to guide the rats to use the unaffected side or affected in the system. It seems the rats can use any side to grasp the end-effector. Please state clearly in the discussion part.

It is now mentioned at the end of section 3.2 that a Plexiglass box can be placed inside the cage to constrain the rat to use either left or right forelimb:
"In the present study the rats used the preferred limb to perform the movements, but this could also be constrained with a Plexiglass box positioned inside the cage in a way to limit window access to the desired limb (Fig. 4)."
The Plexiglass box is also indicated on figure 4 (left diagram). Note that data of 10 rats are now reported in the updated manuscript, and that data from both left-handed and right-handed rats are presented in Figure 6.

Reviewer: 2

This paper introduces an approach for evaluating the possible benefits of incorporating robotics use with rat models. Greater control of experimental variables such as movement kinematics and subject uniformity is cited as the reason for applying an established method of motor rehabilitation for humans to rat models. The potential for using rat models was supported by measures of the rat's familiarization with the robot, in addition to the capability of the robot to alter movement trajectories of the rat models. In a sense, this paper presents the development and early testing of the mechanical side of a much bigger initiative to study neuro-motor recovery from stroke.
The design and implantation, in addition to the task were well outlined. Figure 7 clearly answers the questions presented in the introduction regarding whether rats can be trained to use a robot manipulandum, and whether a 3 DOF robot can instigate motor adaptation in a rat model.
A large problem with this paper lies in the provision of previous animal studies to support the importance of designing this particular system. The authors did not acknowledge the contribution of previous studies using monkeys as an animal model for decoding various attributes of movement and the introduction of force constraints. Failure to recognize the similarity between end-goals of previous studies in monkeys and the study presented in this paper is evident in the statement "Although the motor system of the rat – the animal most often utilized for such studies", which is ambiguous at best.

The introduction has been rewritten. It now includes references to previous studies using monkeys as animal models for detecting the mechanisms responsible for motor adaptation:
"In humans and non-human primates, these limitations were resolved through the application of robotic devices allowing quantitative assessments of movement kinematics and dynamics, as well as the well-controlled and repeatable rendering of external dynamics (force fields) that precisely perturb

movements, thereby challenging motor adaptation (Shadmehr and Mussa-Ivaldi 1994, Scott 1999, Graham et al. 2003). Although this approach allows the investigation of underlying internal models, the detection of functional changes within the human brain is limited to non-invasive imaging methods (Shadmehr and Holcomb 1997, Diedrichsen et al. 2005) and the assessment of basic synaptic mechanisms is restricted to systemic administration of broadly acting neuromodulatory drugs in healthy subjects (Donchin et al. 2002). More invasive methods are achievable in non-human primates (Scott et al. 2001; Prsa et al. 2010, Paz et al. 2005, Li et al. 2001). However, these studies are limited by small experimental sample sizes and the large effort required to train the animal and address ethical concerns."

In Section 4.2 (Preliminary experiments with rats), Key hypotheses need to be re-framed to be an objective test. For example, "can they safely manipulate it in a natural way" is not testable. Describe how you made objective measurements to contend with this problem.

This formulation ("safely manipulate in a natural way") has now been removed from the text, and the two hypotheses investigated (described at the beginning of section 3.3) are both quantifiable, as presented in Figure 5 (for hypothesis (i)) and Figures 6 and 7 (for hypothesis (ii)):
"The objective of the initial experiments performed with healthy rats using the ETH Pattus was to investigate two key hypotheses crucial for future motor learning experiments in rats, being: (i) can healthy rats become familiar with the experimental environment and grasp the end-effector of the robotic manipulandum, as measured by an increasing number of successful touches and (ii) can force fields implemented on the robotic device perturb or shape forepaw kinematics and kinetics during a pulling movement."

The variables used in Equation 1 should be clearly defined for the reader. Why this relation, and what relevance does it have for future exploration?

Equation 1 has been removed, as it does not have relevance for future exploration, and we believe it is now clearly understandable from the text how the maximum pronation and supination torques were determined, i.e. in section 2.1 – Rat forearm dynamics:
"These values were determined based on the study of O'Sullivan and Gallwey which examined pronation and supination torques in human male subjects (O'Sullivan and Gallwey 2002), and downscaled with respect to the mass of the rat, assuming a proportional relationship between mass and achievable torque according to the laws of similitude."

"During sessions 11 to 30, tasks 2, 3 and 4 were trained successively together with other experimental tasks consisting in other types of manipulation, beyond the scope of this paper." Any training study needs to understand why, but more importantly, what happened in training. Otherwise others could never replicate your work (a foundational principle of science). I suggest that you include details of "other experimental tasks."

"Other experimental tasks" referred to different types of manipulation that were also attempted, such as pushing and combinations of pushing and pulling. Nevertheless, in the new version of the manuscript, new experiments with new animals were performed using a standardized protocol, where only the experiments described in section 3.3 (Experimental procedures) were successively implemented. The motivation for each of the implemented tasks with respect to our research questions is now given in each of the subsections of section 3.3.

Maximum force output was determined and stated. However, it is important to mention how much force was applied to the rat in task 3 to keep the rat on a straight path, and during task 4 to perturb the intended movement trajectory.

The new Figure 6C now shows examples of interaction forces for one representative rat over two sessions of Experiment 3 (Pulling 10mm in a haptic tunnel); we also show in Figure 6B the velocity profile over one session of Experiment 4 (Pulling 10mm in a velocity-dependent force field) for the same rat. These figures are now discussed in the discussion section of the paper.

Also, the method of determining the spring constant, k, should be stated.

The spring constant k and damping constant b were empirically determined to be 0.75N/mm and 0.0002Nsec/mm respectively, in order to achieve stable interaction. This is now mentioned in section 3.3.3 (Pulling 10mm in a haptic tunnel):
"Spring constant $k = 0.75N/mm$ *and damping constant* $b = 0.0002N \cdot sec/mm$ *were empirically determined to achieve stable interaction."*

The mean time required for the rat to touch the end-effector is very subjective. The standards and methods used to determine mean time should be briefly explained.

This represents the time between the moment when the robot reaches the start position in front of the cage and the moment the end-effector is touched by the animal. These results have been removed, and for the new version of the manuscript, new experiments have been performed during which the protocol for task 1 was slightly changed. Details of the task are explained in section 3.3.1 (Touching the end-effector).

Were the animals food-restricted to maintain motivation?

Yes, in order to maintain motivation, the animals were food-restricted. This information was added in section 3.1 (Animals):
"Animals were food-deprived for 24h before the first training session. Subsequently, daily food supplements (40-60g/kg of standard diet adjusted to maintain constant body weight) were given after training. Access to water was ad libitum."

While well written, I recommend a second pass to both economize and to formalize the English. Some of the wordings are a bit casual.

The manuscript has been proof-read by a native English speaker.

Minor comment: It would be beneficial to briefly discuss the variance in velocity or force output and path trajectory between the animals during tasks 2-4 in comparison with healthy human subjects.

Dynamic data collected during experiments with new rats is now presented in Figure 6, where one plot (Figure 6C) shows the force profile over two sessions of Experiment 3 (Pulling 10mm in a haptic tunnel) and one plot (Figure 6B) compares the velocity profiles over one session of Experiment 2 (Pulling 10mm in a null field) with one session of Experiment 4 (Pulling 10mm in a velocity-dependent force field). Discussion on similarities with human subjects has been included in the discussion section of the paper.

Reviewer: 3

This is a very interesting paper and the authors should be commended for this effort to bring a new dimension to the study of motor learning in rodent models as there has been a noticeable lack of limb adaptation studies. It is indeed exciting to see the possibility of mapping the effect of focal lesions on various kinds of motor learning using the robot.

I appreciate that it is a challenge to be both a methodology paper and a results paper but I think a number of things could be done to make this paper better.

1. The manuscript needs to be read carefully by a native English speaker. For example the word "stagnation" in the introduction is not what the authors want to say.

The manuscript has been proof-read by a native English speaker.

2. Use of terminology is loose throughout the manuscript: motor learning, skill, and adaptation are used interchangeably. This is unfortunate as the authors start by trying to distinguish them and then forget that they were so careful later in the manuscript. This is not just a semantic issue. The tasks here are grasping, pulling, haptic guidance, and then a perturbing force field. I assume that tasks 1 and 2 would need to be trained first in all rats - they are shaped to use grasp and then pull. The haptic guidance constrains the limb to a channel whereas perturbing force task causes a velocity-dependent lateral displacement. In essence, all four tasks are learning tasks but the authors do not explain the choice of tasks or the different kinds of learning that they involve. It seems that the first 3 involve some kind of reinforcement learning whereas the authors think the 4th will require adaptation. This may be, but it is also possible that the kind of learning done in tasks 1 and 2 (and perhaps 3) will influence how the rats learn task 4. Regardless, more explanation/discussion about the chosen tasks is required.

Extensive parts of the manuscript have been rewritten, including section 1 (Introduction) where the terms of motor skill learning and adaptation are introduced, as well as section 3 (Experimental validation) where a better and clearer explanation for the choice of the 4 experiments is provided. We further comment in section 5 (Discussion and outlook) on the data recorded during these experiments and discuss similarities with human subjects in terms of motor learning and adaptation.

3. The authors list many benefits of the robot including that it can create many kinds of learning task and it can quantify movements. In terms of kinematics, the robot only provides end-effector data not joint angles. In terms of dynamics - no actual measurements are given in the paper, which is disappointing. Could the authors comment?

Yes, this is true; the robot provides end-effector data only, not joint angles. We collect kinematic and kinetic data only at the endpoint (referred as "forepaw kinematics and kinetics" or "endpoint kinematics and kinetics" in the manuscript). However, this could be complemented by video recordings, which is the purpose of adding an HD webcam over the workspace, to acquire data of the manipulations taking place within the specific area. This is now clarified in the text in section 4 (Results) and section 5 (Discussion and outlook). Regarding the actual measurements that the robot can record, we have now added kinetic data in the new Figure 6, where two plots report data on velocity (Figure 6B) and force (Figure 6C) measurements during Experiment 3 and Experiment 4, respectively.

4. The behavioral data in the 4 rats needs some comment. Inspection of figure 5 shows that R2 jumped to near maximal performance in one session. Similarly R3 has a sudden jump at session 9. The same could perhaps be said for the other two rats. Thus it appears that task 1 leads to an "aha" moment in the rat. Figure 6 is similar - for example R# only changes in sessions 9 and 10.

Data were collected with new rats, for which the protocol was slightly modified and standardized (see section 3.3 (Experimental procedures)). This time, during Experiment 1 (Touching the end-effector) the rats were specifically instructed (lured) to perform the specific task, instead of waiting for the animal to realize on its own that touching the end-effector leads to a food reward. In this way, we eliminate the "aha" moment, and rats were able to successfully achieve the task within 3 training sessions. New group data presented in Figure 5 shows this improvement.

5. It is not clear how learning is quantified for task 3 - if the trajectory is limited to the haptic tunnel then the trajectories of course will have a smaller error. To show learning it would be necessary to turn off the haptic tunnel and still see smaller deviations from the straight line than in task 2. No learning is quantified for task 4.

We thank the reviewer for this comment. Data related to this point have been added in the new version of Figure 7, which now quantifies the influence of the haptic tunnel by showing retention after the haptic tunnel is turned off. Comments are now addressed in section 5 (Discussion and outlook). Nonetheless, it should be noted that the purpose of this paper is not to show motor learning in rats, but rather to demonstrate the usability of our robotic platform for such motor learning studies in the future.